# EXHIBIT 2

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## Mapping of a type 1-specific and a type-common epitope on the E2 (gp53) protein of bovine viral diarrhea virus with neutralization escape mutants.

Deregt D, Bolin SR, van den Hurk J, Ridpath JF, Gilbert SA.

Animal Diseases Research Institute, Canadian Food Inspection Agency, Lethbridge, Alberta.

Bovine viral diarrhoea viruses (BVDV) have recently been segregated into two genotypes, BVDV 1 and BVDV 2. However, the antigenic differences and similarities of BVDV 1 and BVDV 2 remain poorly defined. In this study, the E2 epitopes of two neutralizing monoclonal antibodies (mAbs) produced against an isolate of BVDV 1 were mapped. The mAb 157, previously determined to be broadly cross-reactive to BVDV, was discovered to be BVDV 1-specific, whereas mAb 348 bound to and neutralized BVDV 2. Both mAbs bound to epitopes within the first 192 amino acids of the E2 protein as determined by reactions with a C-terminally truncated E2. To identify critical amino acids affecting these epitopes, mAb escape mutants were selected for sequencing from BVDV 1 and BVDV 2 strains with different (wild-type) mAb binding phenotypes. In addition, the E2 gene of several BVDV were sequenced and the sequences were compared with amino acid changes in mutant viruses. Single nucleotide changes in escape mutants selected with mAb 157 resulted in deduced amino acid changes at E2 positions 9, 32 or 72. Amino acid changes at position 72 also affected the epitope of mAb 348. Alignment of E2 nucleotide sequences revealed that BVDV 2 are missing six nucleotides encoding the equivalent of amino acids 31 and 32 of BVDV 1 and thus, this difference can account for the BVDV 1-specificity of mAb 157. Single nucleotide mutations in mAb 348 escape mutants of BVDV 1 and BVDV 2 resulted in changes in 3 amino acids in the previously described immunodominant 71-74 region (Virology 190, 763-772). A fourth amino acid change observed in a mutant of BVDV 2 extended this region to position 77. Thus, the amino acid changes affecting the conserved epitope of mAb 348 occurred in a short spatial array over only seven amino acids, unlike the described composite epitopes previously mapped to this region.

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